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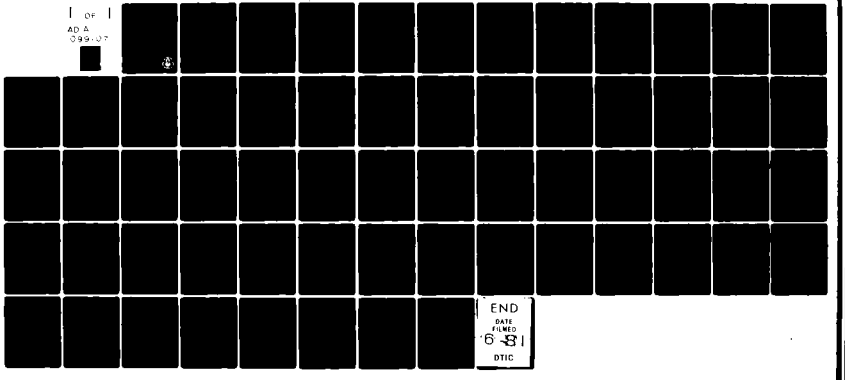

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TECHNICAL REPORT 81-

LEVEL II

THE DETERMINATION OF PHYTOTOXICITY

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This report indicates a format, acceptable procedures, and suitable test plants for determining phytotoxicity of compounds used and/or released to the environment by the United States military. The objective of this report is threefold: (1) to be a guideline for developing phytotoxic protocols, (2) to define protocols that can be used in determining phytotoxicity in the laboratory and in the field, and (3) to be an information source on phytotoxicity.

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PREFACE

Both domesticated and wild species of plants serve many functions in the natural environment; they provide food for man and animals, erosion control, fiber products, maintenance of ecological communities, and aesthetic beauty. Destruction of plants through contamination with phytotoxicants can lead to severe economic losses and result in changes in communities affecting man and animals. Past, present, or future activities of the U.S. Army could pose serious phytotoxic hazards through release of chemical compounds to the environment. Control of these hazards requires the identification of those compounds that are phytotoxic and a determination of concentration levels that impose unacceptable risks.

The objective of this report is threefold: (1) to be a guideline for developing phytotoxic protocols, (2) to define a protocol that can be used in determining phytotoxicity, and (3) to be an information source on phytotoxicity. It is expected that this report will be used by U.S. Army organizations in determining any phytotoxic properties of military-related compounds that may be released to the environment.

This report was prepared in part during the summer of 1978 while the author served an appointment under the Intergovernmental Personnel Act of 1970 at the U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD, and in part while he served as consultant in plant physiology to this Laboratory thereafter. Final editing was performed at the Franklin Research Center, Philadelphia, under Contract DAMD17-79-C-9129 (M. Hall).

PROTOCOL DEVELOPMENT

INTRODUCTION

Many organic and inorganic substances are phytotoxic. That is, these substances can disrupt normal growth and development processes in plants and cause such abnormalities as shape and coloration changes, chlorotic and necrotic spotting, yield reductions, population shifts, and plant death. However, not all compounds are phytotoxicants, and the degree of phytotoxic activity of a given concentration differs for different compounds, making some acceptable for controlled release into the environment with no harm or minimal harm to plant life.

Controlled, selective release of compounds in order to limit injury to plants depends upon an accurate evaluation of the phytotoxic activity of a compound. The phytotoxicants must be distinguished from nonphytotoxicants, and relatively safe limits of phytotoxicants within defined environments must be established for construction of release guidelines.

APPROACH

Phytotoxic activity of a compound is generally determined by placing the suspected compound in contact with test plants and observing the formation of any injury during subsequent growth and development. If these test plants show signs of injury, reduced growth, or altered development compared with control plants, the test compound is a phytotoxicant. The relative phytotoxic activity and safe maximum limits for phytotoxicants within the environment are determined by growing test plants in association with various concentrations of the phytotoxicant and selecting the concentration that gives a defined percentage of phytotoxic injury to the test plants as well as the concentration below which no phytotoxic injury occurs.

Injury to vegetation has been used to screen herbicides and to indicate soil and atmospheric conditions for many years.[1,2] The ability or inability of specific plants to grow and develop normally while in contact with chemical compounds is well established as a quick, valid, and valuable test for phytotoxic materials[1,3] with severity of phytotoxic injury related to the concentration of the phytotoxicant and the relative phytotoxicity of the compound.

With identification and interpretation, phytotoxic injury signs can be and have been used to distinguish phytotoxicants from nonphytotoxicants and to indicate the presence, type, and concentration of phytotoxicants within the plant's environment.[4,5] Specific injury symptoms have become identified with specific types of compounds.[4-6]

CONSTRAINTS

In the evaluation of compounds for phytotoxic activity through growth and development of indicator plants in a medium containing the suspected

phytotoxicant, there are several phases which may limit the accuracy of the evaluation (Table 1). Failure to understand and account for limitations could lead to mistaken conclusions.

TABLE 1. PHASES IN TESTING A COMPOUND FOR PHYTOTOXIC ACTIVITY

1. Collection of test sample	6. Application of test sample to indicator plant
2. Handling of test sample	7. Recognition of phytotoxic signs
3. Storage of test sample	8. Quantification of phytotoxic signs
4. Selection of indicator plants	9. Data analysis
5. Growth of indicator plants	10. Interpretation

An accurate evaluation of the phytotoxicity of a compound or the air, water, and/or soil at any location begins with the procedure used for sampling the compound or location. Only those samples that truly contain and represent the compound or location in question are acceptable. Contaminants present in samples may be phytotoxicants, and thus results would falsely indicate phytotoxic activity of the suspected compound. Nonrepresentative samples may indicate no phytotoxic activity where phytotoxicants actually exist.

Proper handling and storage of suspected phytotoxic material is necessary to assure purity and stability of the test samples. Treatment of test samples in a manner that destroys the identity and original concentrations of suspected phytotoxicants would give false results.

Indicator plants representative of the plants in the contaminant release area must be selected and grown in an environment that will show sensitivity through phytotoxic signs. Monitored abnormalities in growth, development, and/or physiology may be the result of phytotoxicant effects on metabolic processes or of physical injury to cells. Phytotoxicity screening tests monitor morphological and physiological abnormalities, not biochemical changes. Depending upon the phytotoxicant, the environmental conditions, and the susceptibility of the test plant, abnormalities may appear shortly after delivery of the phytotoxicant to the plant or sometime later as the plant continues to develop.

An accurate phytotoxic evaluation using test plants as biological indicators requires the suspected compound to be in contact with the plant tissue and depends on the sensitivity of metabolic systems in test plants and the ability of the investigator to recognize phytotoxic signs. Contact of the suspected phytotoxicant with the test plant, physical form of the suspected

phytotoxicant, and susceptibility of the test plant population should resemble natural conditions for positive comparison to the ambient environment. Any factor that disrupts test requirements can lead to misinterpretation and misapplication of results.

Meeting test requirements is sometimes difficult. In the ambient environment, plant contact with suspected phytotoxicants could occur along several pathways and in several forms (Figure 1). Similarly, plants themselves exhibit differential species and varietal sensitivity to phytotoxicants and may only be sensitive to phytotoxicants under specific situations ranging from a sensitive growth or development phase to specific environmental stress conditions that are not duplicated under test programs. Subtle alteration in competition or reproductive succession in native plant communities may be observable only over several successive growth seasons.

Using seedling plants precludes gaining information on compound phytotoxic activity at later stages of plant development processes, such as flowering, seed formation, fruit ripening, cold-hardiness, and senescence. Using mature plants precludes gaining information on compound phytotoxic activity in earlier stages of development processes, such as seed germination, leaf formation, and pigmentation. Similarly, since screening tests are generally for relatively short time periods, there is no immediate way of determining if the compound is a chronic or acute phytotoxicant.

TEST DEVELOPMENT

A precise test protocol in which all conditions affecting phytotoxic test results are controlled probably is beyond current technology because of the large variation in susceptibility to phytotoxicants within the plant kingdom and the range of ambient environmental conditions that can affect susceptibility. These considerations require that a series of procedures be established to determine phytotoxic or nonphytotoxic activity under specific conditions. The total effort devoted to testing for phytotoxic activity under various conditions will be limited by the availability of resources and by the acceptability of the risk that a compound found to be nontoxic is indeed phytotoxic under some (untested) conditions.

A test protocol must be adaptable to a wide range of situations and test locations, yet standard enough to allow comparison among different tests. Guidelines for a standardized phytotoxic activity test are presented in Table 2. All phytotoxic activity tests are to be conducted with adequate sample size and replication to provide valid statistical confirmation of observations.

A phytotoxic activity test consists of four parts: the indicator plants, the test compounds, the interaction of plants and compounds, and the observation of phytotoxic signs. Development of a valid phytotoxic activity test is determined by type, form, and manner of these four elements. Criteria for checking the validity of a phytotoxic activity test are listed in Table 3. All phytotoxic activity tests compare treated plants to control plants under the same conditions, except that controls are not exposed to phytotoxicants.

TABLE 2. GUIDELINES FOR STANDARDIZED PHYTOTOXIC ACTIVITY TEST[a]

1. Can be conducted by technical level personnel
2. Equipment and instrumentation requirements should be relatively simple, inexpensive, and readily available
3. Suitable for use in testing a wide variety of compounds and plants
4. Results should be applicable to ambient conditions
5. Necessary cost and time commitments should be low
6. Procedures are defined for accurate reproducibility of tests

a. Adapted from Rubinstein et al., 1975.[5]

TABLE 3. CRITERIA FOR VALID PHYTOTOXIC ACTIVITY TEST USING INDICATOR PLANTS[a]

1. Defined test conditions
2. Plant response to phytotoxicant increases with an order related to dose
3. Within limits of sample variation, phytotoxic responses are reproducible

a. Adapted from Freed 1964, p. 41.[7]

Plant material used as indicator plants in phytotoxicity tests must be defined in order to allow comparison from one test to another and to the environment where potential phytotoxic compounds would interact with plants. Growth and use of plants in a diseased or stressed condition can lead to misinterpretation of test results or differences in plant sensitivity to phytotoxicants. Generally, more than one type of plant is used to give a more complete test by accounting for differences in plant susceptibility. Table 4 lists guidelines to be used in selection of indicator plants.

TABLE 4. GUIDELINES FOR SELECTION OF INDICATOR PLANTS IN PHYTOTOXIC ACTIVITY TESTS

1. Representative of species in contaminant area
2. Representative of diversity in plant kingdom
3. Availability of seed stock at reasonable cost
4. Relatively rapid growth and development
5. Limited, nonspecific requirements for growth and development
6. Compatibility to growth conditions of other indicator plants
7. Representative of important economic crops
8. Suitable for identification and quantification of phytotoxic injury
9. Known history of susceptibility to phytotoxicants

Because potential phytotoxic compounds can exist in many physical and chemical forms, their application in the phytotoxic activity tests must conform to that expected within the natural environment. Thus, information on the physical and chemical fate of suspected phytotoxicants after release to the environment is necessary. Disappearance of phytotoxicants could occur by such processes as leaching, volatilization, adsorption, decomposition, or metabolism and thus make them of low phytotoxic hazard. Different concentrations of compounds are applied to establish estimated levels of 50 percent phytotoxic response and a threshold level below which no phytotoxic response occurs. Guidelines for application of test compounds to plants are listed in Table 5.

The determining factor in whether a compound is a phytotoxicant and at what concentrations a compound becomes a phytotoxicant depends to a large extent on the nature of the physical interaction between compound and plant. The several forms and pathways of compound contact with plants are illustrated

TABLE 5. GUIDELINES FOR APPLICATION OF TEST COMPOUNDS TO INDICATOR PLANTS IN PHYTOTOXIC ACTIVITY TESTS

1.	Compound of defined purity and concentration
2.	Compound in form expected within contaminant area
3.	Compound applied to indicator plants at time and in manner of expected interaction with plants in contaminant area

in Figure 1. Some compounds could cause phytotoxic injury through simple surface contact with plant tissue, whereas others may need to be absorbed into the interior of the plant cells. Compounds that are phytotoxic when applied to plant surface tissues may be nonphytotoxic as root-absorbed and translocated compounds.

Of special importance is the concentration at which contaminants are placed in the plant's environment. Many compounds that show no phytotoxic activity at low concentrations can be expected to cause injury to plants at higher concentrations. Therefore, it must be decided in advance whether the purpose of a test for phytotoxic activity is to determine (1) the concentration of the compound that will cause defined injury or (2) if injury will occur at a preselected concentration.

Any phytotoxic effect evidenced is dependent upon the availability of the phytotoxicant to reach the site of phytotoxic action in the plant. Since there are several pathways by which a compound can arrive at the location of plants in the field (Figure 1), the most likely pathways must be considered. Compounds should be thoroughly incorporated into soil or supplied as irrigation water, as dust or droplets to leaf surfaces, as vapors, or in other forms and manners required to mimic the natural interaction pathway.

Plant material must be observed regularly for evidence of phytotoxic signs. Specific phytotoxic signs that could appear on plant tissue cannot be predefined, but investigators should be aware of previously observed phytotoxic signs (Table 6) and quantitatively and descriptively record any differences between control and treated plants at each level of applied phytotoxicants. When feasible, color photographs are taken of control and injured plants to record and preserve the injury signs for comparison to previous or future studies.

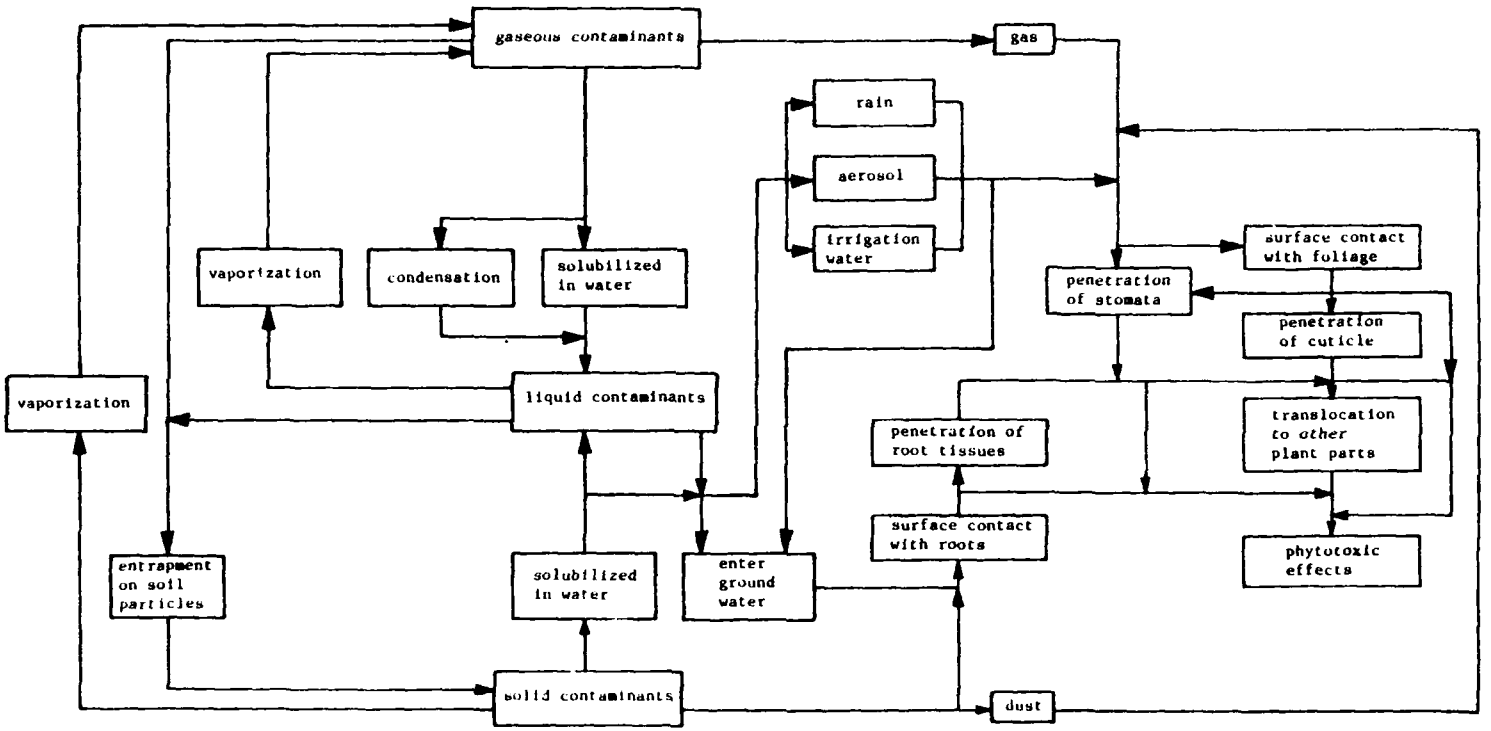

Figure 1. Forms and pathways of phytotoxicant contact with plant tissue.

TABLE 6. EXAMPLES OF PHYTOTOXIC SIGNS

Condition	Definition
Abnormal brace roots	Short, stubby brace roots
Abnormal pigmentation	Color development in leaf not usually associated with species development
Abnormal veination	Altered pattern of leaf vein development
Abscission	Loss of leaves; loss of flowers
Adventitious root initiation	Development of roots on stems
Branched ears	Branches on normally unbranched ears of grains
Brittleness	Development of stem tissue that is easily broken
Bunched ears	Ears with increased number of spikelets at each node
Change in flower numbers	Increased or decreased quantity of flowers per inflorescence
Chlorosis	Loss of chlorophyll in leaves and/or stems
Crinkling	Retardation of vein growth causing mesophyll tissue to bulge out between veins; failure of monocot leaf to emerge from sheath properly; loss of smooth margin on dicot leaves
Cupping of leaves	Turning of leaf edges up or down
Death	Plant dies
Desiccation	Drying of leaf tissue
Elongated stems	Increased length of stems resulting from increased elongation of cells
Epinasty	Bending down of petioles
Ethane production	Increase in ethane production by injured plant tissue
Fasciation	Band-shaped distortion of normally cylindrical organ

TABLE 6 (Cont.)

Condition	Definition
Feathering of leaves	Elongation of leaves
Flecking	Chlorotic or necrotic spots scattered throughout the leaf
Fusion of leaflets	Abnormal growth pattern of leaf where individual leaflets are united
Galls and/or tumors	Tissue growths on stems due to disorganized cell division
Hypocotyl swelling	Enlargement of hypocotyl
Interveinal chlorosis	Loss of chlorophyll between veins of leaves
Marginal chlorosis	Chlorosis of leaf edges
Marginal necrosis	Necrotic tissue along edge of leaves
Mesophyll reduction	Leaf development where mesophyll tissue between veins is not formed
Metabolite changes	Increases or decreases in DNA, RNA, protein, or other metabolite within cells
Misshaped petals and sepals	Broadening or narrowing of petals or sepals
Mottling	Randomly located chlorotic areas in leaves
Nastic curvature	Permanent or temporary twisting of stems or petioles generally involving actively growing tissue due to unequal rates of elongation on different sides of the stem
Necrosis	Local areas of dead tissue occurring on leaves, stems, roots, fruit, or flowers
Negative geotropism	Roots grow up rather than down
Opposite spikelets	Spikelets in grains opposed to one another instead of alternate
Plant population changes	Alteration in percentage of species within a location; reduced numbers of certain or all species

TABLE 6. (Cont.)

Condition	Definition
Pigmentation changes	Increase or decrease in chlorophyll, anthocyanins, betacyanins, xanthophylls, or other pigments
Reduction or multiplication of flower parts	Decrease or increase in number of flower parts
Reduced germination	Percentage of seed germinating is lower than controls
Reduced growth	Growth of plants or plant parts is less than control
Respiration changes	Increase or decrease in CO_2 evolution or O_2 consumption
Root branching	Prolific production of side roots generally from main roots in dicots and adventitious roots in monocots
Root hair stunting	Shortening and thickening of root hairs, sometimes characterized by swelling at root hair tip
Root thickening	Short thick roots or localized sections of roots caused by inhibition of elongation growth and expansion in lateral growth
Sex change of flowers	Increased production of female flowers in plants having imperfect flowers
Shortened stems	Decreased length of stem resulting from decreased elongation of cells
Stamens or carpels changes	Reduced or increased number of stamens and/or carpels; fusion of carpels; flattening of stamens
Stem cracking	Development of longitudinal cracks along stem
Sterility	Inability of flower to produce fertile seed
Stress ethylene	Production of ethylene gas by injured plant tissues

TABLE 6. (Cont.)

Condition	Definition
Stunting	Decreased growth in plant
Suppressed root hair production	Decrease in quantity of root hairs on roots
Suppressed root growth	Decrease in root growth as evidenced by reduced length
Thick stems	Extension of stem in lateral direction generally associated with decrease in longitudinal growth of cells and/or increased cell division in cambium
Thickening	Increased thickness of leaves
Transpiration changes	Increase or decrease in water movement through stomata
Tubular initiation	Formation of tubers on stolons or in leaf axils
Tubular leaves	Fusion of leaf rims to form funnel or cup-like leaves that may or may not encircle the stem
Tweaked ears	Arrangement of grass culms where portions of rachis are devoid of spikelets
Veinal chlorosis	Loss of chlorophyll at veins of leaves
Veinal necrosis	Necrosis along leaf veins

PHYTOTOXICITY PROTOCOL

INTRODUCTION

A protocol for ascertaining the phytotoxic activity of environmental contaminants is described. The procedures outlined require a series of different tests, some prerequisite to others, depending on the information required. Each portion of the protocol is designed to provide essential data for a clear and consistent phytotoxic evaluation of contaminants, individually and, if required, in combination. The tests outlined are based on three approaches: a field evaluation, use of standard indicator plants, and use of specific tests for specific applications.

The decision pathway for use of the protocol is illustrated in Figure 2. Each successive step is a more complete phytotoxicity test with associated tests designed for those concerns particular to one type of phytotoxicant, plant, or location. Progression in or to subsequent tasks is decided on the basis of test results, program needs, and resource availability.

Initial task selection is based on whether the contamination is predispersed and on the probable mode of distribution of the phytotoxicant (Figure 3). Additional tasks are selected by considering the phytotoxicants and the plant population of the contaminant area. Long-lived and water-soluble compounds require more thorough phytotoxic testing than short-lived or insoluble compounds. More thorough phytotoxic tests are required where the threatened target plant population includes food and feed plants. Indications of compound phytotoxic activity from prior studies or especially susceptible plant populations may dictate selection of specific tests.

Developing a general application protocol for a definitive identification of all phytotoxic contaminants is probably impossible because of variability in plant sensitivities among different species during growth and development and under different environmental conditions. Seemingly innumerable combinations of plants and contaminants could occur under a multitude of environmental conditions. This protocol is designed to test reasonable chances of a contaminant being phytotoxic, as measurable by current field and laboratory capabilities.

GENERAL PROTOCOL

All primary screening tests for phytotoxicity are conducted using the standard test plants of corn, Zea mays L. var. 'Butter and Sugar'; oats, Avena sativa L. aestivum var. 'Clintford'; beans, Phaseolus vulgaris L. var. 'Black Valentine'; and radish, Raphanus sativus L. var. 'Scarlet Globe.' The reasons for selecting standard test plants are outlined in Table 7, and the reasons for selecting these specific plants for primary screening tests are presented in Table 8. Additional test plants should be added to the screening test if there are special reasons to test them, such as being the dominant plant

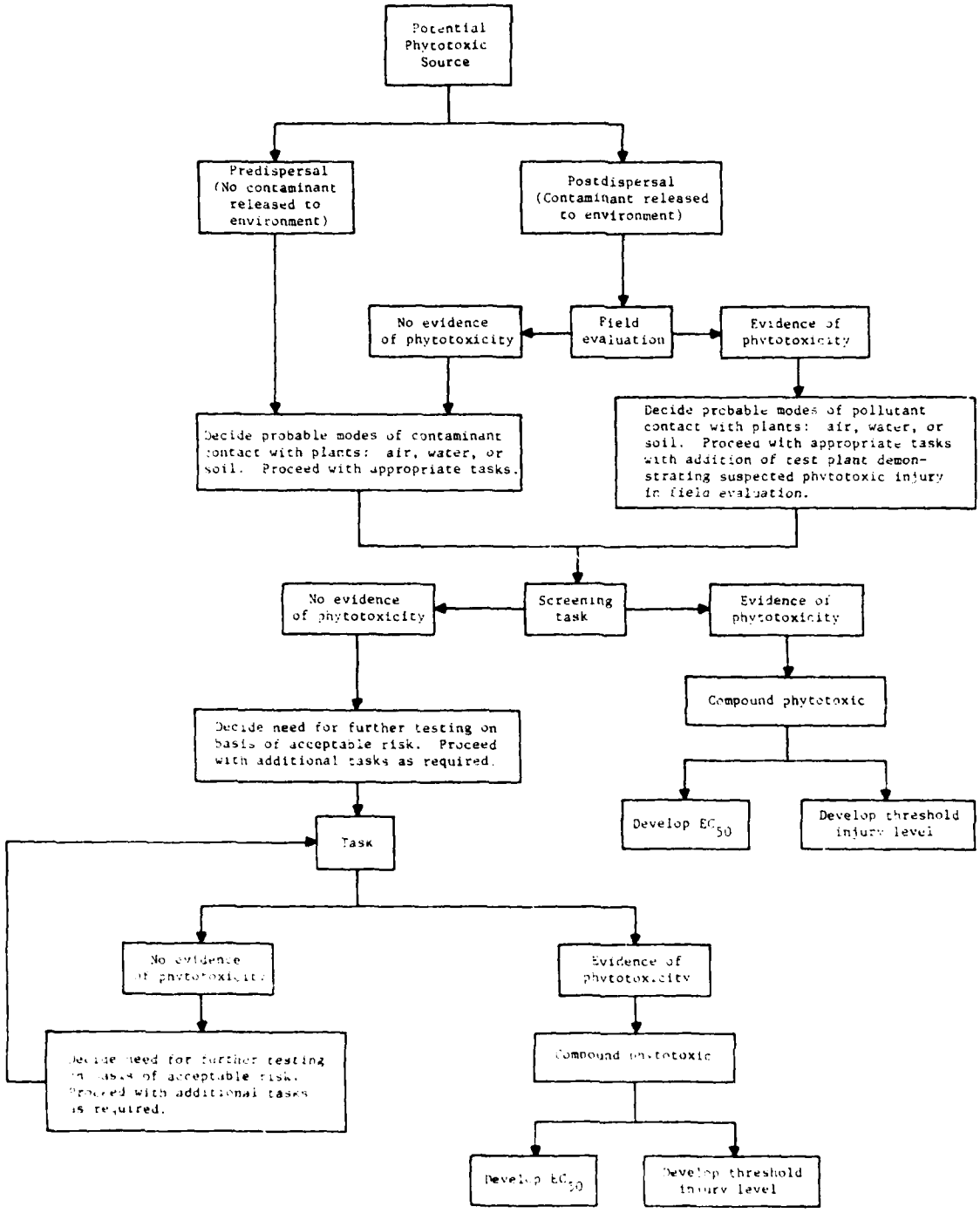

Figure 2. Decision pathway for determining compound phytotoxicity.

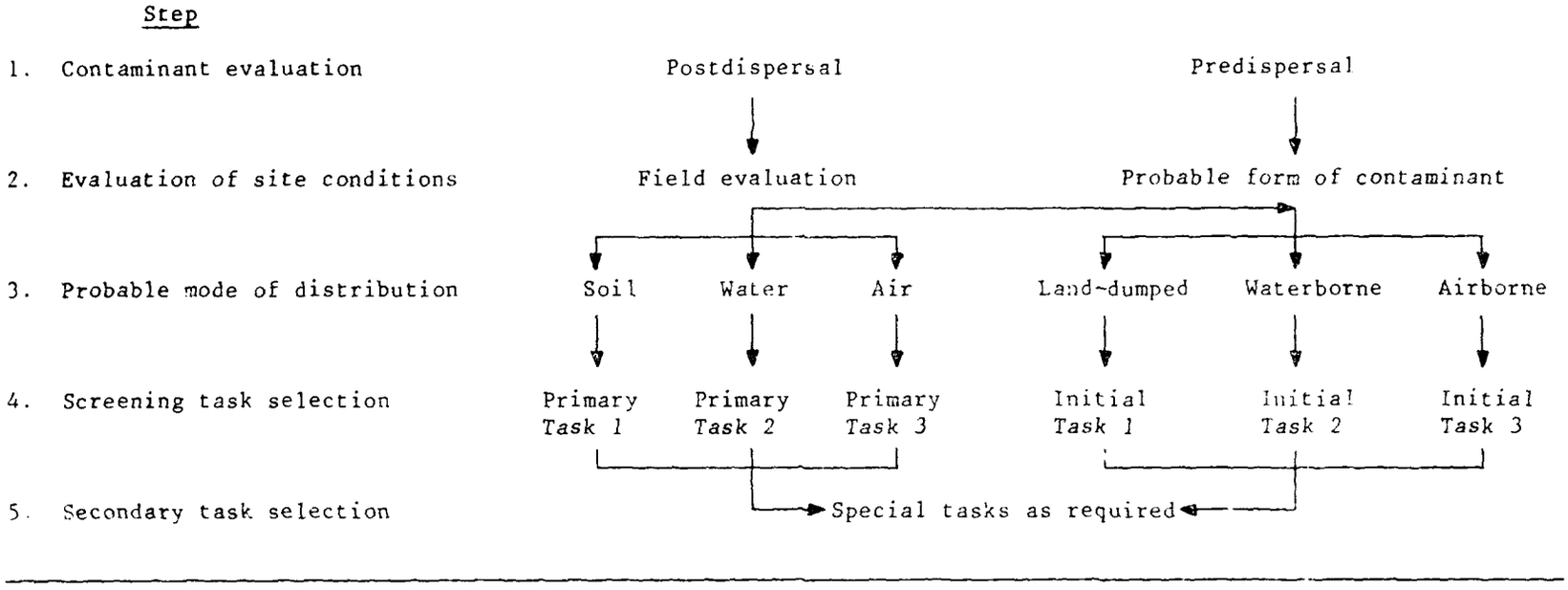

Figure 3. Task selection.

species in an evaluation area, being a plant species of considerable social or economic importance to an area, or being a plant species observed to be sensitive to phytotoxicants in field evaluations.

TABLE 7. REASONING FOR SELECTION OF STANDARD INDICATOR PLANTS

1.	Enable comparison of results from different test locations
2.	Develop reference table of relative phytotoxicities among compounds
3.	Ease management of phytotoxic activity tests

TABLE 8. PLANTS TO BE USED AS STANDARD INDICATOR TEST SPECIES

Plant	Reasons for Selection
Corn, Zea mays L. var. 'Butter and Sugar'	Seed readily available, adaptable to controlled environment growth, monocot, C-4 photosynthetic metabolism, suitable for yield trials, economically important, used in previous phytotoxic activity screening tests
Oat, Avena sativa L. aestivum var. 'Clintford'	Seed readily available, adaptable to controlled environment growth, monocot, C-3 photosynthetic metabolism, suitable for yield trials, economically important, used in previous phytotoxic activity screening tests
Bean, Phaseolus vulgaris L. var. 'Black Valentine'	Seed readily available, adaptable to controlled environment growth, dicot, suitable for yield trials, economically important, used in previous phytotoxic activity screening tests
Radish, Raphanus sativus L. var. 'Scarlet Globe'	Seed readily available, adaptable to controlled environment growth, dicot, root crop, rapidly growing, suitable for yield trials, economically important, used in previous phytotoxic activity screening tests

Plants in the germinating and seedling stage are used for screening tests (Table 9). In general, plants are observed from seeding until 3 weeks' growth has occurred. Observations determine alterations in germination, growth, or abnormal markings. All plants are grown under environmentally controlled conditions (including, but not limited to, adequate moisture, light, mineral nutrients, temperature, CO_2, O_2, and freedom from stresses such as pollution, diseases, and insects) to assure good and vigorous plant growth. Table 10 lists suggested growth environments.

TABLE 9. REASONING FOR SELECTION OF SEEDLINGS AS TEST PLANTS

1.	Rapidly growing plants are generally more susceptible to phytotoxicants
2.	Requirements of facilities and support media for growth are minimal
3.	Short growth periods enable phytotoxicity tests to be quickly repeated a relatively large number of times
4.	Environmental requirements are generally less specific than for more mature tissue
5.	Changes in germination and growth easily quantified

All phytotoxic activity tests are conducted with adequate sample size and replication to provide statistical confirmation of observations. Since phytotoxic injury is determined by comparison of treated and control plants, all conditions, except for exposure to phytotoxicant, must be identical for control and treated plants. Statistical analysis follows the guidelines referenced in the section on information on phytotoxicity (p. 27).

The range of concentrations of any suggested phytotoxic compounds applied to test plants depends on the purpose of the phytotoxic activity test, such as determining (1) the concentration level at which plant injury occurs or (2) if injury occurs at maximum levels expected within the environment. In the second instance, initial concentrations of compounds, as a minimum, should be two times the expected ambient concentration. If injury occurs at this level, lower concentrations are tested to relate level of injury to concentrations and to determine the threshold level below which no injury occurs. In any event, the highest concentration of test compound used would be that at which death of plants or other defined injury occurs. Control plants have no test compound applied.

TABLE 10. GROWTH ENVIRONMENT FOR STANDARD TEST PLANTS[a]

Climatic Factors	Desired Condition
Temperature	Minimum day 20°C; minimum night 10°C; maximum 28°C. Note: oat seeds may need to be prechilled at 5°C for 5 days before planting
Light	Sunlight or mixture (4:1 watts) of fluorescent and incandescent artificial light; intensity 12,000 lux; duration 12-14 hours per day
Relative humidity	10-60%
Carbon dioxide	As per normal air concentration
Oxygen	As per normal air concentration
Edaphic Factors	
pH	6.0 to 7.0
Nutrients	Adequate mineral nutrients for plant growth
Moisture	Daily watering of soil to assure adequate moisture for plant growth[b]
Texture	Suitable for penetration and growth of roots without distress
Temperature	Same as air temperature
Oxygen	As per normal soil concentrations
Other Factors	
Biologic	Disease-free, pest-free
Pollution	Pollution-free
Physical	Stress-free

a. Test plants should have uniform growth environment. Test plants can respond differently to phytotoxicants as the growth environment varies.

b. Excessive watering could lead to leaching of water-soluble test compounds and soluble nutrients (i.e., nitrogen) from soil and/or concentration of compounds at the top or bottom of pots.

Plant material is examined regularly for evidence of phytotoxic injury. Specific phytotoxic signs that could appear cannot be prestated, but investigators should be aware of previously identified phytotoxic signs (Table 6). Observations on test plants are recorded. When feasible, color photographs of plants with visual phytotoxic signs are taken. All injuries are quantified.

FIELD EVALUATION

The objective of the field evaluation is to discern any indications of phytotoxic activity of predispersed compounds on native flora at field contaminant concentrations. The best evidence of contaminant phytotoxic activity is observation of definable plant injury in the field. Results of this study help select test plants for other tasks outlined in this protocol and may indicate the extent of the contaminant problem.

Experimental

Selected indigenous plant species of each area exposed to contaminants are examined for phytotoxic symptoms by on-site inspections for phytotoxic injury signs (Table 6) or by remote sensing of plant stresses (Appendix C). A reporting form suitable for use during field diagnosis of phytotoxic injury to plants is presented in Figure 4.

Observations must be completed during a plant's growing season so that phytotoxic signs will be more easily seen. At least two visits to the contaminated area, one in spring after trees have leaves and a second visit approximately 4 to 6 weeks later, are preferable. Plants in the immediate contaminant source area and at points away from the source are observed until dispersion calculations indicate significant dilution of pollutants. In addition, adjacent but uncontaminated areas are selected as control plantings to indicate normal plant growth and development.

Color photographs are made of all injury signs for comparison with phytotoxic signs produced in other tasks. All affected plants are identified.

Conclusions

Phytotoxic signs at source sites may indicate the presence of phytotoxic compounds among released contaminants. Injury may be in the form of reduced yields, population modifications, or other nonobservable changes in the field. Lack of phytotoxic signs does not preclude phytotoxicity of compounds. Concentration of compounds may be too low to produce phytotoxic signs.

Field Evaluation for Phytotoxicity

Location:

State_____ Military Post_____

County_____ Specific Site_____

Town_____

Observations:_____

_____

_____

_____

Plant species affected_____

Land area involved_____ No. of plants_____

Data of incidence and/or observation_____

Avg. % of Each Plant Affected: 0-10, 11-30, 31-60, 61-80, 81-90, 91-99, 100

Avg. % of Plants Affected: 0-10, 11-30, 31-60, 61-80, 81-90, 91-99, 100

Loss in (check one): Quality_____ Quantity_____

Remarks_____

_____

_____

Date_____ Observer's Name_____

Figure 4. Exemplary Reporting Form*

*Adapted from Waddell, T.E. and D.G. Gillette

PRIMARY TASK 1. SOIL AT POSTDISPERSAL CONTAMINANT AREA

The objective of the first screening task is to determine if soil at a contaminant postdispersal location contains phytotoxic substances in a concentration that will induce a plant response.

Experimental

Representative samples of soil from the contaminated location of concern are secured for testing purposes. Soil samples should weigh approximately 2 kilograms (dry weight) and be maintained in a manner to preserve any phytotoxicants. The soil sample is thoroughly mixed, screened to remove large stones, and adjusted to a pH between 6 and 7 unless changing the soil pH will cause loss of potential phytotoxicants. A subsample of soil is placed in a suitably sized container (approximately 1,000 grams of soil per container at an approximate depth of 10 centimeters) and watered and fertilized as needed to provide adequate moisture and plant nutrition (Table 11). Test plants are seeded in rows (at least 10 plants per row) in the soil, and the container with soil and seeds is placed in a greenhouse or controlled environmental chamber for germination and growth of plants for 3 weeks. Control plants are treated in the same way, except they are grown in a soil sample from a noncontaminated area.

All plants are observed periodically for germination, growth, and development of phytotoxic injury signs. After 3 weeks' growth, plants are removed from soil, and their top and root systems are examined for phytotoxic-induced developmental abnormalities or lesions.

Conclusions

Phytotoxic signs on treated plants that are not found on control plants may indicate the presence of phytotoxic contaminants within soil samples. Lack of phytotoxic signs does not preclude phytotoxicants in soil. Injury in the form of reduced yields or population modification is observable only during long-term studies. Concentrations of compounds may be too low to produce phytotoxic signs.

PRIMARY TASK 2. WATER AT POSTDISPERSAL CONTAMINANT AREA

The objective of this task is to determine if water sources at a contaminant postdispersal location contain phytotoxic substances in a concentration that will induce a plant response.

Experimental

Representative samples of water from the contaminated location of concern are secured for testing. Water samples are about 5 liters and maintained in a manner to preserve any phytotoxicants. Test plants (at least 10 each of each

TABLE 11. DESIRABLE CHARACTERISTICS OF SOIL FOR USE IN PHYTOTOXICITY TESTS[a]

Constituent	Guidelines
pH	6.0-7.0[b]
Available phosphorus	25-75 ppm[c]
Exchangeable potassium	100-150 ppm
Exchangeable calcium	750-1,500 ppm
Exchangeable magnesium	150-250 ppm
Available nitrogen	Sufficient for plant growth without deficiency signs[d]
Other nutrients	Additional sulfur, boron, iron, and zinc may be necessary in some soils, especially sandy soils and soils low in organic matter
Electrical conductivity (EC)	$\leq$ 4 mmho[e]
Sodium adsorption ratio (SAR)	$\leq$ 13 $meq^{1/2}$ $L^{-1/2}$[e]

a. Adapted from Lorenz and Bartz 1968, p. 338.[9] Soils in shallow containers may have drainage problems. Care should be taken not to overwater. Long-term experiments may require dilution with silica sand (80 parts sand:20 parts test soil) to improve plant-soil water relations.

b. This represents a desirable pH range; however, any pH $\geq$ 4.5 and $\leq$ 8.5 could be acceptable. As pH is changed, availability and thus phytotoxicity of many soil-contained compounds could change. Soil at pH extremes may require use of acid- or alkaline-tolerant test plants.[10]

c. For neutral and calcareous soils ($NaHCO_3$-P). At pH $\leq$ 6.3 measured with Bray's acid-NH_4F, extraction of P will give somewhat lower values (W.D. Guenzi, USDA-SEA, Fort Collins, CO. Personal communication).

d. Generally, a blanket application of nitrogen can be made to soil, 25 mg N/kg soil.

e. From Bolt and Bruggenwert 1976.[11]

species) are seeded in rows on washed sand in suitable containers that hold approximately 1,000 grams of sand at a depth of 10 centimeters and are grown under hydroponic conditions using a plant nutrient solution (Table 12) made up with the water samples. Seeded tests are placed in a greenhouse or controlled environmental chamber for germination and growth of the plant for 3 weeks. Control plantings are treated in the same way, except nutrient solution is made from noncontaminated water.

If water at the contaminant site of concern can be used as irrigation water, the effect of the test water on foliage is determined. Large droplets of the test water sample are applied to foliage of 2-week-old test plants.

All plants are observed periodically for germination, growth, and development of phytotoxic injury signs. After 3 weeks' growth, plants are removed from sand, and their root systems are examined for phytotoxic-induced developmental abnormalities or lesions.

Conclusions

Phytotoxic signs found on treated plants and not on control plants indicate the presence of phytotoxic contaminants in the water. Lack of phytotoxic signs does not preclude phytotoxicants in water. Injury in the form of reduced yields or population modification is observable only during long-term studies. Concentrations of compounds may be too low to produce phytotoxic signs.

PRIMARY TASK 3. AIR AT POSTDISPERSAL CONTAMINANT AREA

The objective of the third screening task is to determine if air at a contaminant postdispersal location contains phytotoxic substances in a concentration that will induce a plant response.

Experimental

Twelve- to 14-day-old test plants (at least 10 of each species) grown in soil in containers of suitable size to allow for unstressed growth are placed downwind and as close to the source of the contaminant as possible while still allowing for plant growth. The plants are left on location for a minimum of 24 hours under conditions to ensure active growth and open stomata. Control plants are maintained similarly but in an uncontaminated area.

Following exposure to contaminants, plants are returned to previous growth conditions for 48 hours. Observations are made of development of phytotoxic injury signs on the aerial portion of the plants.

Conclusions

Phytotoxic signs found on treated plants and not on control plants indicate the presence of a phytotoxic contaminant in the air. Lack of phytotoxic signs does not preclude the phototoxicant in the air. Injury to

TABLE 12. PLANT NUTRIENT SOLUTION FOR INDICATOR PLANTS[a]

Salt	Solution (g/liter)
$Ca(NO_3)_2 \cdot 4H_2O$	1.18
KNO_3	0.51
KH_2PO_4	0.14
$MgSO_4 \cdot 7H_2O$	0.49
$FeC_4H_2O_6$	0.005
H_3BO_3	0.0029
$MnCl_2 \cdot 4H_2O$	0.0018
$ZnSO_4 \cdot 7H_2O$	0.00022
$CuSO_4 \cdot 5H_2O$	0.00008
$H_2MoO_4 \cdot H_2O$	0.00002

a. From Hoaglund and Arnon 1968.[12]

plants may come only after prolonged exposure and/or at different times in plant development when the plant is more susceptible. Concentrations of compounds and exposure may be too low to produce phytotoxic signs.

INITIAL TASK 1. PREDISPERSAL OF LAND-DUMPED CONTAMINANTS

The objective of this study is to evaluate the possible phytotoxicity of compounds placed on or incorporated into soil.

Experimental

Soil representative of the disposal site is secured for testing purposes; the volume of soil must be large enough for a replicate set of test evaluations. Soil is air-dried, thoroughly mixed, screened to remove large stones, and--unless alteration of pH or addition of nutrients would adversely modify the solubility, availability, or solubility of the test compounds--adjusted to a pH between 6 and 7; suitable amounts of plant nutrients are added to support vigorous plant growth (Table 11). Samples of soil (approximately 1,000 grams from a depth of up to 10 centimeters) are collected for growth of treated or control plantings. The compounds to be tested are incorporated into soil in any manner adequate to ensure uniform distribution

of the test compound throughout the soil sample. Soil containing the test compound and the control nontreated soil are placed in separate containers and watered to provide adequate moisture for seed germination.

All plants are observed periodically for germination, growth, and development of phytotoxic injury signs. After 3 weeks' growth, plants are removed from soil, and their root systems are examined for phytotoxic-induced developmental abnormalities or lesions.

Conclusions

Phytotoxic signs found on treated plants and not on control plants indicate that the contaminant added to the soil is phytotoxic. Lack of phytotoxic signs does not preclude the added contaminants being phytotoxic. Injury in the form of reduced yields or population modification is observable only during long-term studies. Concentrations of compounds may be too low to produce phytotoxic signs.

INITIAL TASK 2. PREDISPERSAL OF WATERBORNE CONTAMINANTS

The objective of this study is to evaluate the possible phytotoxicity of compounds solubilized or dispersed in water.

Experimental

Water representative of the disposal site is secured for testing. Desirable characteristics of water are presented in Table 13. The volume of water must be large enough for a replicate set of test evaluations. A plant nutrient solution (Table 12) is made using the water and is divided into two subsamples. The contaminant is added to one sample to ensure thorough distribution of the contaminant throughout the water sample. The noncontaminated solution serves for control plantings. Test plants (at least 10 of each species) are seeded in rows on washed sand in suitable containers that hold approximately 1,000 grams of sand at a depth of 10 centimeters and are grown under hydroponic conditions using the plant nutrient solutions made previously. Seeded tests are placed in a greenhouse or controlled environmental chamber for germination and growth of the plant for 3 weeks.

If water at the contaminant site of concern can be used as irrigation water, the effect of the test water on foliage is determined. Large droplets of water containing contaminant are applied to foliage of 2-week-old test plants.

All plants are observed periodically for germination, growth, and development of phytotoxic injury signs. After 3 weeks' growth, plants are removed from soil, and their root systems are examined for phytotoxic-induced developmental abnormalities or lesions.

Conclusions

Phytotoxic signs found on treated plants and not on control plants indicate the contaminant added to the water is phytotoxic. Lack of phytotoxic signs does not preclude phytotoxics in soil. Injury in the form of reduced yields or population modification is observable only during long-term studies. Concentrations of compounds may be too low to produce phytotoxic signs.

TABLE 13. DESIRABLE CHARACTERISTICS OF WATER FOR USE IN PHYTOTOXICITY TESTS[a]

Constituent	Guidelines
Salinity	
Electrical conductivity	<0.75 mmho/cm
Permeability	
Electrical conductivity	>0.5 mmho/cm
Adjusted sodium absorption ratio	<6.0
Specific ion toxicity	
From root absorption	
Sodium (evaluate by SAR)	<3
Chloride	<4 meq/liter; <142 µg/liter
Boron	<0.5 µg/liter
From foliar absorption	
Sodium	<3.0 meq/liter; <69 µg/liter
Chloride	<3.0 meq/liter; <106 µg/liter
Other	
NH_4-N + NO_3-N	<5 µg/liter for sensitive crops
HCO_3 (with foliar application)	<1.5 meq/liter; <90 µg/liter
pH	normal range 6.5-8.4

a. Adapted from Ayers and Branson 1978, Table 19.[13]

INITIAL TASK 3. PREDISPERSAL OF AIRBORNE CONTAMINANTS

The objective of this task is to evaluate the possible phytotoxicity of compounds added to the air. An airborne substance could be a gas, aerosol, or dust.

Experimental

Test plants (at least 10 of each species) are grown in soil in containers of suitable size to allow for unstressed growth of test plants. Growth conditions, including soil pH and fertility, must ensure development of vigorous and healthy test plants. Following 12 to 14 days' growth, test plants are enclosed in a suitable test chamber and exposed to the airborne contaminant for a minimum of 2 hours. Test conditions must ensure the plants are actively growing with sufficient light and moisture to maintain open stomata on leaves during exposure to the contaminants. Control plants are treated similarly, except they are not exposed to contaminants.

Following exposure to contaminants, plants are returned to previous growth conditions for 48 hours. Observations are made of any development of phytotoxic injury signs on the aerial portion of the plants.

Conclusions

Phytotoxic signs found on treated plants that are not on control plants indicate that the airborne compound is phytotoxic. Lack of phytotoxic signs does not preclude the compound being a phytotoxicant. Injury to plants may come only after prolonged exposure and/or at different times in plant development when the plant is more susceptible. Concentrations of compounds and exposure times may be too low to produce phytotoxic signs.

INFORMATION ON PHYTOTOXICITY

INTRODUCTION

This section presents information and comparison data for use in studying phytotoxicity. Designating a compound as phytotoxic is relative to the concentrations of the compound and the susceptibility of the plants. The information in this section is to help distinguish phytotoxicants from nonphytotoxicants.

A positive finding in a well-conducted phytotoxicity study is strong evidence that a designated compound is a phytotoxicant. However, a negative finding does not prove conclusively that a compound is not a phytotoxicant since many factors can obscure a positive association between the environmental pollutants and the induction of phytotoxic signs on plants. One must constantly be aware of this "risk" factor and maintain control of all tests through selection of identical test plant populations, exposure to varying levels of the compound of interest, and expression of defined results

from specific phytotoxicant tests. Some guidelines for declaring a compound a phytotoxicant (Table 14), a phytotoxicity rating guide (Table 15), and an indication of the level of phytotoxicity of some common herbicides (Table 16) are presented.

CONFLICTING DATA

Divergent results sometimes occur between phytotoxic activity tests. Occasionally, some species may show phytotoxic signs in a test while others do not, or plants within a species may be injured by a given concentration of phytotoxicants in one test and not in another. In these cases, all tests should be reviewed, and poorly designed or poorly conducted tests should be discarded. If appropriate test procedures were followed, it is generally best to accept the positive phytotoxicant tests until further testing can be completed. Differences in sensitivity among plant species or cultivars within a species are not unusual. Differences in the growth environment (temperature, light, soil nutrient) may lead to differences in plant sensitivity among phytotoxic activity tests.

SYNERGISM AND/OR ANTAGONISM

Some compounds may become phytotoxic or nonphytotoxic in the presence of other compounds. Mixtures of two phytotoxicants have been shown to produce more and/or different injury to plants than either phytotoxicant alone.[14,15] Although no specific tasks have been outlined in this report for testing the above possibilities, situations in which mixture of compounds may exist could require testing. Mixtures of compounds may be used in any of the screening tests or appropriate combinations of screening tests can be adapted for use (see the section on Phytotoxicity Protocol).

STATISTICAL ANALYSIS

Phytotoxicant testing methods discussed in this report offer a probability that a specific compound or series of compounds could be phytotoxic to various plants under certain conditions at specific concentrations. To properly evaluate the tests and determine injury thresholds, the data must be examined and an appropriate statistical analysis completed to determine the probability that any observed phytotoxic signs could have been due to chance alone. Appropriate guidelines for statistical analysis of phytotoxicity tests have been outlined in another publication.[16]

CHARACTERIZATION OF RISK

Any determination and declaration of a compound as a phytotoxicant involves certain important assessments. For example, what are the ecological and economic costs of deciding a compound is not or will not be a phytotoxicant under conditions in the field when indeed it is; or what are the clean-up and protection costs of declaring a compound a phytotoxicant when it is not?

TABLE 14. GUIDELINES FOR DECLARING A COMPOUND A PHYTOTOXICANT

1. Review all data with specific attention to experimental design, conduct, and interpretation of results

2. Regard short-term initial screening tests as valuable but insufficient evidence for definitive identification of phytotoxicants or injury thresholds

3. Regard the outcome of a single test as potentially the result of chance variation and accept only data from appropriately designed and analyzed experiments

4. Proceed only when the available evidence indicates a compound is a phytotoxicant at a concentration that could occur within the environment

TABLE 15. PHYTOTOXICITY RATING GUIDE

Phytotoxic Concentrations (threshold level for initiation of phytotoxic signs)		
Phytotoxicity rating	Air or Water (ppm)	Land (kg/ha)
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
Moderately toxic	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

TABLE 16. RELATIVE PHYTOTOXICITY OF SOME HERBICIDES AS EVIDENCED BY RECOMMENDED APPLICATION RATES

Herbicide	Application Rates[a] (kg/ha)
2,4-D (2,4-dichlorophenoxy)acetic acid	0.28-2.24
Trifluralin α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	0.56-1.12
Dalapon 2,2-dichloropropanoic acid	0.84-44.8
Atrazine 2-chloro-4-ethylamino-6-isopropylamine-s-triazine	2.24-44.8
Picloram (Tordon) 4-amino-3,5,6-trichloropicolinic acid	2.24-9.6
Amitrole 3-amino-1H-1,2,4-triazole	2.24-10.1
Erbon 2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate	134-192
Sodium borate	243-1,230
Sodium arsenite	2[illegible]

a. Data from Berg (1974)[17] and Berg (1980).[18]

The following assessments are recommended:

1. Consider the level of phytotoxic action indicated by a compound in field evaluations and/or phytotoxicity tests. Risks are generally greater for those compounds for which plants have a low injury threshold or when a variety of test plant species are susceptible.

2. Consider the pathway by which plants will be exposed to the phytotoxicants. Disposal of phytotoxicants via air or water will probably lead to larger areas of contamination and thus injury to more plants. In some instances it may be relatively easy to restrict distribution of phytotoxicants to a specific location or to a time when plants are not susceptible.

3. Consider the amount of the compound to be released to the environment. Even compounds of low phytotoxic action may injure large quantities of vegetation where the dose to the plants is high.

In addition, there are undoubtedly social and political considerations in the amount of phytotoxicity testing that must be done before a compound can be declared phytotoxic or nonphytotoxic. This report does not characterize those assessments.

SAFETY PROCEDURES

All personnel working with phytotoxicants should be briefed on safe handling and application of phytotoxicants prior to any work tasks. Some phytotoxicants may be hazardous to human health. Improper disposal of phytotoxicants used in testing procedures may contaminate other soil and plants, expanding the phytotoxic hazard. The following procedures are recommended during:

1. Use and application

   - Wear appropriate protective clothing during all handling procedures.

   - Avoid personal exposure through use of good laboratory and greenhouse practices.

   - Wash with soap after handling toxic materials.

   - Be sure that all containers and plant containers are empty and clean when application is completed.

   - Maintain records of type of compound used.

   - Post signs to notify others of hazards.

   - Be alert for symptoms, perspiration, breathing difficulties, or other symptoms that may indicate a health hazard from personal exposure.

2. Disposal of phytotoxicants, contaminated soil, and plant material

   a. Collect all phytotoxicants and contaminated material

   b. Package for disposal in tightly sealed containers

   c. Label all material indicating potential hazards

   d. Follow instructions of military contract officer for disposal by high temperature incineration or another designated procedure.

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APPENDIX A

CONVERSION FACTORS

SOIL

1 cu ft muck	=	25 to 30 lbs dry
1 cu ft clay and silt	=	68 to 80 lbs dry
1 cu ft sand	=	100 to 110 lbs dry
1 cu ft loam	=	80 to 95 lbs dry
1 cu ft average soil	=	80 to 90 lbs dry
1 acre foot (43,560 cu ft)	=	3,500,000 to 4,000,000 lbs
Soil surface plow depth (6 inches)	=	2 million lbs (1,000 tons) per acre

Volume of compact soil increases about 20% when tilled

ppm in soil	=	mg/kilogram (i.e., 1 ppm = 1 mg/kilogram)
100 lbs of substance/acre	=	approximately 50 ppm; 0.035 oz/sq ft; 1 g/sq ft
1 kg/hectare (ha)	=	0.8923 lb/acre
1 lb of substance/acre	=	approximately 0.25 tsp/100 sq ft

LAND AREA

1 ha	=	10,000 sq m = 2.47104 acres
1 sq rod	=	1 perch = 272.5 sq ft
1 acre	=	160 sq rods = 43,560 sq ft = 0.404687 ha
1 sq mile	=	1 section = 640 acres
1 township	=	36 sections = 23,040 acres

Note: Parts of Texas are surveyed in labors (177.5 acres) and leagues (25 labors).

WATER

1 gallon	=	8.355 lbs
1 cu ft	=	7.48 gallon = 62.42 lbs
1 acre inch	=	113 tons (approximately) = 102.3 m^3
1 acre foot	=	43,560 cu ft = 323,136 gallons
ppm in water	=	µl/liter (i.e., 1 ppm = 1 µl/liter)
ppm	=	(eq wt) x (m eq/liter)
ppm dissolved solids	=	approximately 640 x mmho/cm conductivity
0.1 oz of substance/gallon of water	=	approximately 800 ppm; 0.6 lbs/100 gal; 0.08% solution
1 pint of substance/acre	=	approximately 0.25 tsp/100 sq ft

GASES

For conversion of:

ppm to $\mu g/m^3$

$$\mu g/m = \frac{ppm \times MW \times 10^3}{MV}$$

$\mu g/m^3$ to ppm

$$ppm = \frac{\mu g/m^3 \times MV \times 10^{-3}}{MW}$$

where MW = molecular weight

MV = molecular volume = 24.46 liters/mole at 25°C, 760 mm Hg

SMALL UNIT CONVERSIONS[a]

Soil Unit	Soil (cc)[b]	Amount of Substance (mg) Needed for 1 lb/6 in Acre Equivalent[c]
Standard pots		
3-inch	180	0.117
4-inch	500	0.325
5-inch	900	0.585
6-inch	1,500	0.975
7-inch	2,400	1.560
8-inch	3,785	2.461
Short pot, 8-inch	2,900	1.885
Pan, 8-inch	1,400	0.912
Liter	1,000	0.649
Gallon	3,785	2.461
Cubic foot	28,317	18.410
Bushel	35,238	22.909

a. Adapted from Smith, F.F. 1952. Conversion of per-acre dosages of soil insecticide equivalents for small units. J. Econ. Entomol. 45:339-340.
b. Volume of pots or containers used for soil will vary. This table is to be used as a guideline only.
c. Based on soil bulk density of 1.3. For testing, calculations should be on a weight basis and not volume. This table is to be used as a guideline only.

LENGTH

1 kilometer (km)	=	1,000 m = 0.62137 miles
1 meter (m)	=	39.37 inches = 3.28 ft
1 rod	=	5.5 yds = 25 links
1 chain	=	66 ft = 4 rods = 100 links

MASS

1 kilogram (kg)	=	1,000 grams (g) = 2.204622341 lbs
1 metric ton	=	1,000 kg
1 pound (lb)	=	453.5924 grams

VOLUME

1 cubic centimeter (cc)	=	0.06102338 cu inches
1 cu ft	=	1,728 cu inches = 28,317.016 cc
1 quart	=	0.946333 liters
1 gallon	=	3.785332 liters
1 teaspoon	=	4.93 milliliters
2 cups	=	473.167 milliliters
1 liter	=	2.11342 pints = 1.05671 quarts

YIELD

ton/acre	=	0.446 metric ton/hectare
lb/acre	=	1.121 kg/hectare
bu/acre	=	1.15 hectoliter/hectare

APPENDIX B

OTHER BIOASSAY TESTS

Numerous bioassay tests have been developed over a period of many years to answer general and specific questions on the phytotoxic nature of chemicals. This report does not list all those studies; indeed, any listing would be incomplete, since new tests are continuously being developed. Instead readers interested in certain tests may wish to examine the bibliography (Appendix D) included in this report.

Readily applicable sets of bioassays are included in two reports prepared for the U.S. Environmental Protection Agency:

1. Rubinstein, R., E. Cuirle, H. Cole, C. Ercegovich, L. Weinstein, and J. Smith. 1975. Test Methods for Assessing the Effects of Chemicals on Plants. NTIS PB-248 198.

2. Duke, K.M., M.E. Davis, and A.J. Dennis. 1977. IERL-RTP Procedures Manual: Level I Environmental Assessment Biological Tests for Pilot Studies. NTIS PB-268 484.

Selection of bioassay tests depends upon the need. Specific requirements (plants, environment, test compounds, etc.) may very well require specific tests. For many phytotoxic activity tests, a general test run under a wide range of conditions may be used to indicate compounds as phytotoxicants. Selection of the appropriate test requires judgment by the investigator.

APPENDIX C

REMOTE SENSING OF PHYTOTOXICITY

A technique of detecting and quantifying on-site phytotoxic injury through use of remote sensing would be of special value. Use of satellites or airplanes to describe postdispersal of phytotoxic compounds and their location could quickly indicate the extent and level of phytotoxic injury.

Current investigations have indicated the ability of remote sensing to identify plants subjected to several stresses (water, salinity, disease, and pollution).[1,2,3] The technique appears to lack the discretionary value necessary for positive association of plant injury to specific chemical phytotoxicants.

However, patterns of extended phytotoxicant distribution can be identified through use of remote sensing. Changes in plant population[1,4] and leaf injury[1,3] can be identified where relatively large areas are affected.

Remote sensing for identification of plant injury is a specialized skill requiring image making at specific wavelengths. The specific wavelength used would depend upon the stress being examined. In addition, imagery techniques generally require an accurate "ground truth" for positive identification of sensed data.

For recent information on use of remote sensing for detection of phytotoxicants, one can contact the American Society of Photogrammetry, Falls Church, Virginia.

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APPENDIX D

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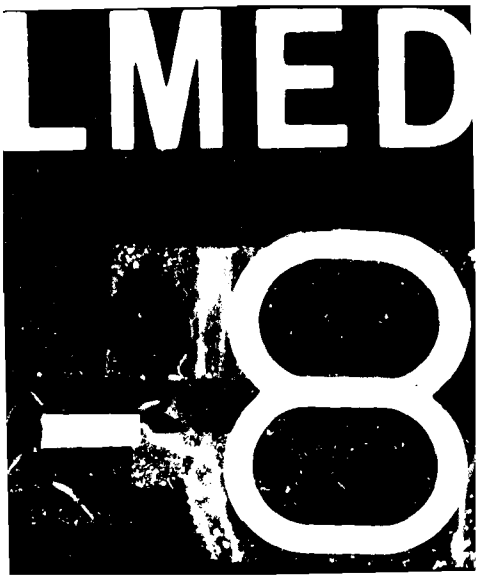
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